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MACULAR BIOACCELEROMETERS ON EARTH AND IN SPACE

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Abstract

Spaceflight offers the unique opportunity to study linear bioaccelerometers (vestibular maculas) in the virtual absence of a primary stimulus, gravitational acceleration. Macular research in space is particularly important to NASA because the bioaccelerometers are proving to be weighted neural networks in which information is distributed for parallel processing. Neural networks are plastic and highly adaptive to new environments. Combined morphological-physiological studies of maculas fixed in space and following flight should reveal macular adaptive responses to microgravity, and their time-course. Ground-based research, already begun, using computer-assisted, 3-dimensional reconstructions of macular terminal fields will lead to development of computer models of functioning maculas. This research should continue in conjunction with physiological studies, including work with multichannel electrodes. The results of such a combined effort could usher in a new era in understanding vestibular function on earth and in space. They can also provide a rational basis for counter-measures to space motion sickness, which may prove troublesome as space voyagers encounter new gravitational fields on planets, or must re-adapt to 1 g upon return to earth.

Introduction

Before humans actually flew in space, there was great fear that space would prove too hostile an environment to withstand. Among the predicted effects of weightlessness were those related to the balance end organs of the inner ear, which are finely tuned to earth's gravitational field. Some medical scientists believed that humans would become severely disoriented and unable to function in space (see Deitlein, 1977). The vestibular system was, therefore, looked upon as a prime candidate for study in space and high priority was given

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to it. These fears, however, did not materialize and interest waned. During the first suborbital flights, when astronauts were closely confined within the vehicle, there were no vestibular-related problems at all. It was only later when spacecraft became larger, permitting movement (particularly of the head), that the syndrome called space motion sickness, or space-adaptation syndrome, appeared. Even then, statistics compiled have shown that only about half the astronauts and cosmonauts become ill, and that the syndrome tends to disappear during the first 2-4 days of flight (Nicogossian and Parker, 1982). It is unclear in the remaining cases whether prophylaxis accounts for some of the resistance to the syndrome, or if some individuals are simply immune. A further interesting fact is that there does not appear to be a relationship between motion sickness in earth's gravitational field and proneness to space motion sickness. Thus, there is currently no means of assessing an individual's inclination to become sick in space through ground-based testing. Upon return to earth, there is a re-adaptation to the 1 g field which varies somewhat in symptoms and in severity from one individual to another (Homick and Miller, 1975; Homick *et al.*, 1977). The period of re-adaptation is roughly proportional to that of the flight (Kornilova *et al.*, 1979) as is also known to occur in sea-sickness. The finding that symptoms of re-adaptation are more universal in astronauts and cosmonauts than are those of space sickness would suggest that all individuals experience an adaptation to weightlessness, whether or not frank illness is a complement of the process.

This paper will consider recent findings which indicate that the vestibular system is most worthy of regaining its place of importance in the NASA space program. The system is highly significant because of its functional organization as a parallel processing, weighted neural network. Such networks are considered to be plastic and highly adaptive to new environmental factors. Maculas, the linear bioaccelerometers of the vestibular system, should, therefore, undergo adaptive changes in response to microgravity. Once adapted, they must re-adapt to earth's gravity, or adapt again to a different, partial gravitational field if that is encountered. This has long-term implications as we look forward to permanent stations on the moon, or to exploration of Mars. Of equal importance is the fact that macular tuning to various gravitational environments can serve as a relatively simple model in the study of central neural adaptation, which must occur as the brain resolves conflicts between visual, kinesthetic and vestibular information in space. Finally, maculas are amenable to computer modelling to predict, simulate, and test adaptive processes.

The Peripheral Receptor as a Parallel Processor

Recent research with long series of sections through rat maculas has shown that type II cells lack an independent innervation to the central nervous system. They synapse with calyces of type I hair cells and with collaterals of calyces (Ross, 1985, 1986). Such synapses were observed first by Ades and Engstrom (1965), although the universality of the arrangement was not appreciated at the time. Much earlier, Lorente de Nó (1926) had described calyceal collaterals in his Golgi studies of mouse maculas, although the existence of two kinds of hair cells was not understood.

The new results shed a different light on the functional organization of the macular receptors. (It should be noted that ampullary end organs appear to have the same kind of

organization, although we have not yet studied them systematically.) Maculas have the functional organization of a data-flow computer that is suitable for parallel processing of information in real-time. What is the basis for this conclusion, and what are the questions raised?

Morphological Findings

Types of Nerve/Terminal Innervation Patterns

Work with montages showed that there are three major kinds of nerve/terminal fields in rat maculas, M, M/U and U, based upon the presence (or absence), and the length, of the unmyelinated pre-terminal segment. These are M-, U- and M/U-types (Ross, 1986).

The M-type essentially lacks a pre-terminal segment, because the nerve is myelinated to the calyx. The nerve does not penetrate the neuroepithelium, but the basal cells and basal lamina closely surround it as it rises to the calyx, which does lie within the macula proper. The myelin of these large-diameter nerves unwraps in an unusual manner at the initial node of Ranvier, situated at the calyx. Some of the leaflets end slightly in advance of the others, so that the portion closest to the calyx has fewer layers of myelin and is also enlarged. One calyx comprises the terminal, which usually contains several type I cells and lacks collaterals.

The U-type is the most complex. The thinly myelinated, smaller diameter nerve loses its myelin below the neuroepithelium and becomes surrounded by processes of the basal cells. The unmyelinated, pre-terminal segment enters the neuroepithelium distal to its actual termination and sometimes hooks around other unmyelinated nerves in its course. It may give rise to a single calyx with subparts, in which case the calyx splits above the cell bodies to follow closely the necks of the enclosed type I cells as they reach towards the heads at the macular surface. In other cases, common in the area of the utricle most completely investigated here, the nerve bifurcates into branches of unequal length. Each branch gives rise to a single calyx containing a single type I cell. Other variations occur, with combinations of calyces with multiple and with single cells not uncommon. In general, calyces of the U-type terminal field have several collaterals, some of which have efferent morphology. Afferent collaterals supply type II cells related to the terminal's field of innervation, and also type II cells more closely related to other terminal fields. Efferent-type collaterals tend to extend to type II cells of the terminal's own field. Particularly in the case of afferent-type collaterals, reciprocal or sequential synapses are common. That is, both a ribbon or spherule junction and a subsynaptic cistern are present in the type II hair cell, and vesicles accumulate near the cisterns on the presynaptic side.

The M/U-type is intermediate with respect to nerve diameter and terminal complexity. Branching of the pre-terminal segment often occurs but collaterals are infrequent. Only those nerves which have short, unmyelinated, pre-terminal segments are categorized as M/U-type.

A different terminology introduced by Goldberg and his colleagues (Goldberg *et al.*, 1985) is based upon the configuration of the calyx alone. That is, whether the terminal has both calyceal and bouton endings (dimorphic), has only a calyceal terminal (calyx), or only bouton endings (bouton units). A further part of this classification is that simple calyces supply individual hair cells and complex calyces supply 2-3 adjacent hair cells.

It will be important in the near future to agree upon one standard terminology that is

meaningful both physiologically and morphologically. In the context of neural networks, we have found the classification by type of nerve/terminal innervation pattern to serve best for development of concepts. The terms M-, U-, and M/U-types bring to mind a particular set of characteristics (outlined above) useful in conceptualizing corresponding physiology. Our morphological findings are that the length of the pre-terminal segment is related to the size of the nerve fiber and its myelination, to opportunity for anatomically close apposition of unmyelinated segments, and to the complexity of the calyceal terminal. (By complexity, we mean the presence of numerous collaterals.) Physiologically, differences in length of a pre-terminal segment imply differences in timing of initiation of response, and size and degree of myelination are reflected in conduction speed of the parent nerve. This is of significance in dealing with the neural networks they form, because network calyces of all 3 kinds of nerve/terminal patterns are addressed by the same type II hair cell in some portions. This would imply chronological encoding of information passed to the central nervous system (see below). Encoding by nerve response characteristics may be reflected in the discreteness or complexity of the terminal field, and in opportunity for electrical coupling of unmyelinated pre-terminals which have long intramacular courses (discussed below).

Computer-assisted 3-D Reconstruction of Macular Nerve/Terminal Fields

Visualization of the hair cells, calyces, and nerves of the maculas has been greatly enhanced by computer-assisted reconstruction of slices through the structures, traced from electron micrographs. The first reconstruction attempted was that of an M-type field, because of its relative simplicity. All of the type II cells synapsing with this type of terminal do so upon the calyx, as no collaterals are present. This simple example required 370 sections to include the calyx and all cells (5 type I, 7 type II) which synapsed with it. The program employed (Kilgore *et al.*, 1984) was capable of illustrating a calyx, type I cells and type II cells individually or in small groupings, but could not show this most simple terminal field in its entirety. Neither was it possible to illustrate kinocilia or stereocilia. The program, while an important first step, simply was not sophisticated enough to handle all the data points required.

Nevertheless, the resulting reconstructions were useful in many ways. They showed, for example, that the type I cells within the calyx did not have identical orientations at their heads. Even in the absence of visualization of the kinocilia and stereociliary tuft, this meant that these cells, at least, did not have identical polarizations. The evidence about type II cell orientations was less clear, because of the inability to construct the entire field with the aid of a computer. The available data did, however, strongly suggest that no two hair cells had identical polarization. A further observation made during the process of reconstruction was that type II cells aggregated into clusters at non-periodic intervals. The clusters usually consisted of 4-7 cells in the region studied, but sometimes only two cells were involved. The cells had closely interdigitating borders, but no synapses were observed.

A further benefit of the reconstructions was the ability to estimate the portion of the macular surface covered by cells innervated by the terminal. For the type I cells, this area is irregular in outline and is ~138 μm long x 25 μm wide. One of the heads of a type II cell lay outside this area, extending the width another ~7 μm at this site. This portion of the macular surface, then, corresponds to the sensory field of the terminal. Its dimensions are very

different from those of the calyx itself which, in this example, is the terminal field since there are no collaterals.

A more complete reconstruction of this terminal and its hair cells has now been accomplished (Fig. 1) with the aid of a different program (Kinnamon, 1986). All of the cells as well as the calyx are visualized in one image, and the kinocilia can be projected in place. This reconstruction verifies the locations and angles of the heads at the macular surface. The angles of polarization of the cells are, however, still too difficult to determine with any reasonable degree of accuracy. Future programs must be devised to slice through the basal bodies of the kinocilia of the hair cells, to resolve this problem.

Fig. 1. This reconstruction illustrates an entire M-type terminal field with 5 type I cells (blue), 7 adjacent type II cells (red), the calyx (green) and kinocilia (white).

Use of the Kinnamon program has permitted us to image examples of all three kinds of nerve/terminal innervation patterns, and to illustrate linkages between terminals. An example of a U-type pre-terminal with a broad calyceal collateral (afferent-type) to a type II hair cell is shown in Figure 2. This interesting pre-terminal split into two branches of unequal length. Each branch ended in a single calyx which held but a single type I cell. Each calyx had 3 type II cells synapsing with it, so that the terminal innervated a total of eight hair cells.

It should be noted that the bifurcating type of pre-terminal was the most common kind observed in network 2 (Fig. 4). Most frequently, each calyx has its own complement of type II cells which do not communicate with the other calyx of the pair, but which do synapse on other, nearby terminals. It is as though each branch has its own sensory field. Sometimes these are close together, as in the example cited here (Fig. 2). In other cases, the two terminals are many microns apart and may cross the striola. In the examples traced thus far, however, the polarizations of the cells communicating with the nerve are not in opposite directions.

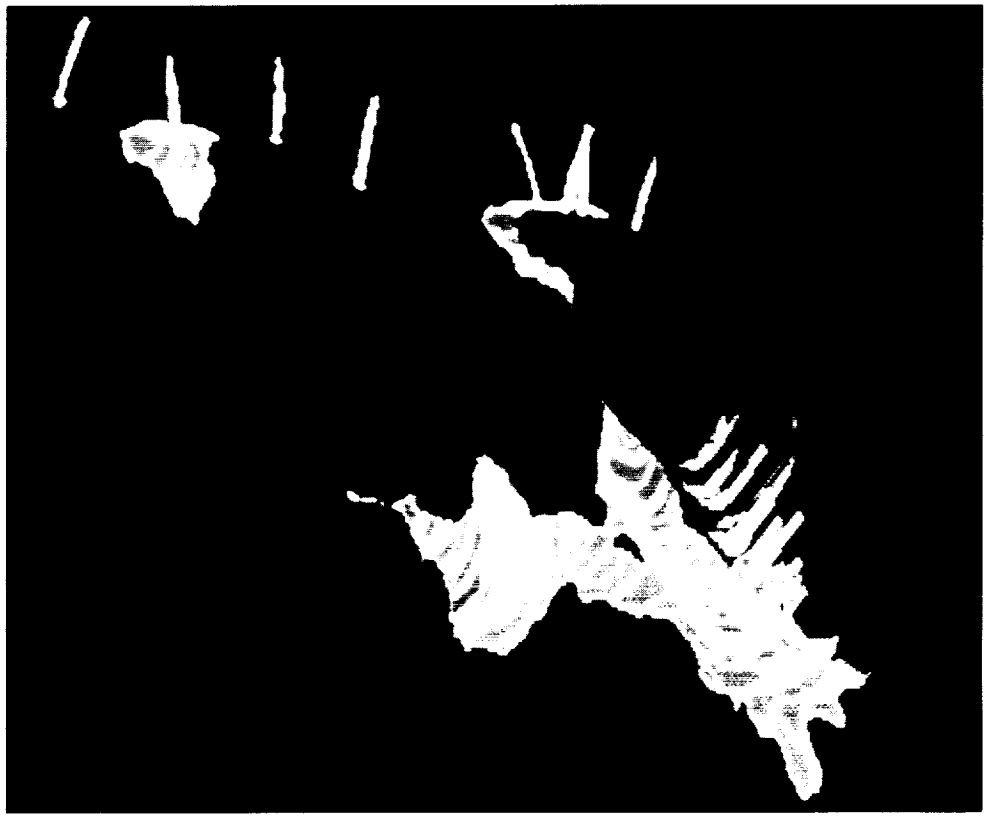

Fig. 2. This reconstruction illustrates a U-type pre-terminal with two unequal branches. One branch has a large calyceal collateral (afferent type) to a type II cell (far right). Each branch has 3 type II cells synapsing with it. Type I cells are blue; type II cells are red; kinocilia are purple and the calyx is yellow.

Neural Network Organization

The organization of the linked terminal fields can be illustrated by 3-dimensional reconstructions, but these rapidly become too large for a small computer to handle. The next stage of this portion of the reconstruction research, which has already begun, entails moving the data points to a workstation capable of more complex reconstructions and rendering them as solids, transparencies, or as slices through the entire field, from various angles.

Another approach is to begin to diagram the linked terminal fields as weighted neural networks, making them interpretable as electric circuits. By weighting is meant, for example, the number and distribution of cells synapsing with specific terminals of the network, the degree of similarity or disparity in angles of polarization of the cells of the sensory field, and the number, kinds and locations of synapses. These factors help determine the direction of information flow in the circuit at any given moment, depending upon the direction and force of the applied linear acceleration.

For the initial stage of this work, we diagrammed two parts of the lateral region of the utricular macula as neural networks, using hair cell number, distribution and linkages. The two parts were selected because of interesting terminals, some of which had been reconstructed. In the first network, connectivity between 67 cells was traced and in the second, 78. A representative part of each network is shown in Figures 3 and 4.

The networks from the two parts of the region examined are different in organization although they proved to be connected. In network 1, all three kinds of nerve/terminal innervation patterns were often linked together (Fig. 3). Surprisingly, network 2 consisted of only U-type nerve/patterns (Fig. 4). There were other interesting differences as well, which also have meaning in neural network theory.

First of all, the ratio of type I to type II hair cells is 1:1.5 in network 1, and 1:2.35 in network 2. Upon examining this finding more closely, it is clear that the 1:1.5 ratio holds because the calyces usually enclose 2-5 hair cells while 2-7 type II hair cells synapse with them along their exterior surfaces. In the case of the 1:2.35 ratio, the calyces enclose fewer type I cells (1-2), but the number of type II cells synapsing with them is most commonly 5. A further extension of these observations is that the average total number of cells synapsing with a terminal varies according to the type of terminal field in network 1. For example, for the region diagrammed, this number is 4 for U-types, 6 for M/U types, and 8 for the M types. In network 2, in which there are only U-type patterns, the average number of cells per terminal is 6.

The results gathered so far indicate that the smallest number of cells in a terminal field is, with rare exception, 4 (one instance of 3 has been found so far). No instances have yet been uncovered in which a single hair cell is innervated by a terminal.

Another observation is that type II hair cell clusters of the region studied here are larger and more predominant in network 1, where they might consist of as many as 7 cells. In network 2, interdigitation of more than two type II cells is uncommon (Figs. 3 & 4).

Finally, the two networks differ in another fundamental way. In network 2, the unmyelinated pre-terminals and their calyces often closely appose one another so that the intercellular space approximates that of a synapse. Such close apposition of these neural structures was not observed in network 1. Close apposition of neural surfaces, including those between type II hair cells, may signify electrical coupling to synchronize activity.

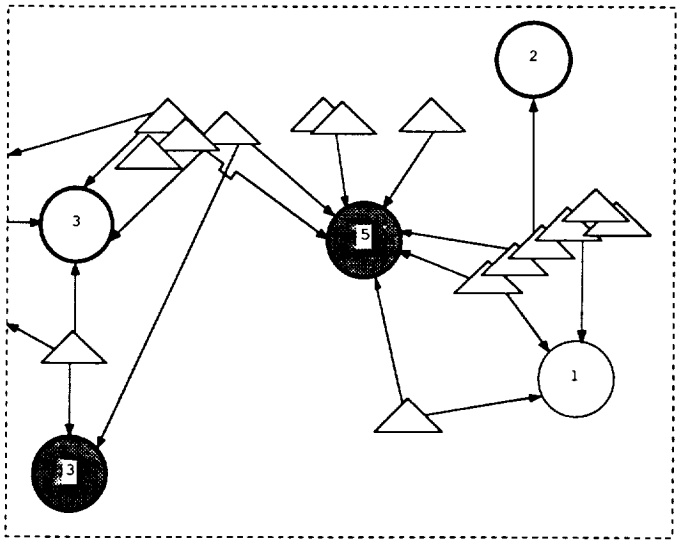

Fig. 3. This diagram is a representative piece of network 1 circuit diagram. Network 1 has all three nerve types linked, large clusters of type II cells, and a type I to type II ratio of 1:1.5. Type II cells are represented as triangles, calyces as circles with the number of contained type I cells noted, and adjacency as arrows.

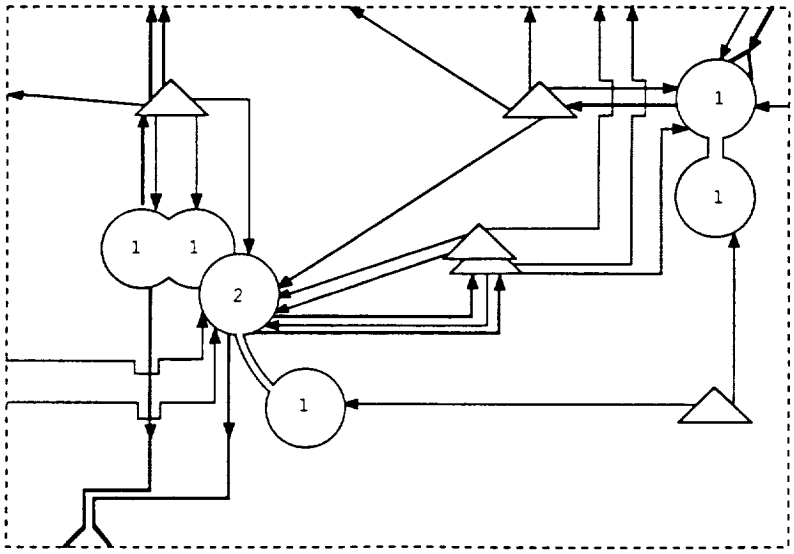

Fig. 4. This diagram is a representative piece of network 2 circuit diagram. Network 2 has only U-type pre-terminals, has close apposition of pre-terminals and calyces, and has fewer and smaller clusters of type II cells than does network 1.

As the research advances in technological capability, further information will be encoded in the circuit diagrams to better illustrate weighting. Noting the distribution, kind and number of synapses will be an important advance. For example, the number of synapses counted in cells reconstructed was 75, with rods prevalent in type I cells and spherules predominant in type II cells. This number does not represent the total junctions present, because only every fifth section was used for reconstruction purposes. Three-dimensional reconstructions using every section will be necessary to study this aspect of weighting in the neural circuits.

Nevertheless, sixty-one of the 75 synapses were in type II cells and, of this number, 50 were spherules. There were five examples of double junctions and serial or reciprocal junctions were not uncommon. Interestingly, 7 of the 14 synapses counted in type I cells occurred in the M-type field illustrated in Figure 1. These numbers emphasize that, in keeping with the high number of interconnections required for distributed processing, type II cells have many more synapses than type I cells. They also demonstrate that there may be weighting differences in synapses according to kind of terminal field. Lastly, they point to some need to know whether there are physiological differences between rod and spherule forms of synapses. One report indicated that spherule junctions are sensitive to catecholamines, at least in frog (Osborne and Thornhill, 1971), suggesting differences in sensitivity to neuromodulators.

Conclusions

The morphological observations clearly indicate that macular bioaccelerometers have a very sophisticated organization into weighted neural networks. The networks are in continuity with one another but differ in functional organization according to location. It is possible that further patterns will emerge as the study is continued to other macular parts. The reason for the relative complexity of some regions compared to others is unclear. The end organ only has to resolve direction and magnitude of linear accelerations; why are differing kinds of networks required to accomplish the job?

One possible answer is that there is enormous redundancy built into this natural parallel processor, so that it will remain a functioning unit even though parts of it are lost through attrition. Redundancy may be a hallmark of natural neural networks that could help explain their long lives, efficiency and adaptiveness.

A related question is why some nerves are regularly firing and some are irregular (Goldberg *et al.*, 1985). There is no precise relationship between calyceal or dimorphic units, the terms employed by Goldberg and his colleagues, and regularity or irregularity of discharge (Goldberg *et al.*, 1985). It would seem, however, that a plausible explanation exists if one studies the composition and organization of the neural networks described here. There is a potential for electrical coupling between calyces, between calyces and pre-terminals, and between pre-terminals in network 2 that is lacking in network 1. This could contribute to the smoothing out of neural responses in the unmyelinated nerves that intertwine, so that they are regularly firing. Because nerve pre-terminals and terminals of network 2 lack such close anatomical relationships, even though some U-type innervation patterns are included, their nerves would follow the patterns of firing initiated by the sensory/terminal network.

The findings presented here, then, argue for the concept that the morphological organization of the nerve innervation patterns and the relationships between components of the neural network are important in determining firing patterns. This point of view differs from that of Goldberg *et al.* (1985), who propose that postsynaptic recovery functions are a major determinant.

Because maculas are neural networks, it is clear that new approaches to the study of their physiology will be required. Means should be found to study nerve activity in adjacent nerves, and in nerves sharing information with the same type II hair cells. Advances in development of multi-channel electrodes, particularly if they will also permit injection of different dyes for later tracing of terminals, will be important. It must be kept in mind, however, that linked nerves do not necessarily enter the neuroepithelium adjacent to one another, particularly in the case of pars externa. A further, most critical need is for reconstruction of terminals of nerves of known physiological response, to learn whether the relationship between hair cell polarizations and specific directional sensitivity (Loe *et al.*, 1973) is linear.

It is clear that much more co-ordinated research involving morphologists, physiologists, physicists, electrical engineers, and neurochemists will be required to factually resolve the issues raised. In particular, a common terminology must evolve that will have clear meaning for the morphologist, physiologist, and neural network theoretician.

It is in this sense that a concerted NASA effort could be most beneficial to the vestibular community, by organizing and supporting an integrated effort to understand information processing in the vestibular receptors through appropriate ground-based research that will lead to a computer model of the weighted neural networks. NASA has the computer ability and expertise to make a significant contribution to neural network theory as well as to vestibular understanding, if its technological capability can be harnessed to a dedicated, investigative, research community. Such a concerted attack will be required if we hope to understand processes underlying neural adaptation to conflicts in signals induced by novel sensory environments, including space, or the unique gravitational fields of the moon and Mars. The findings should have the side benefit of providing a rational basis for devising counter-measures for space adaptation syndrome and for impaired vestibular function that may be particularly debilitating to those humans returning from long voyages in space.

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